Mars Walking Simulation: An Electromyographic Analysis

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ABSTRACT

Context. With a long duration return mission to Mars on the horizon, we must learn as much about the environment and its influence on the musculoskeletal system as possible to develop countermeasures and mitigate deleterious health effects and maladaptation. **Aims.** To determine the influence of simulated Mars gravity on the activity of four locomotor muscles while walking, in comparison to 1 G, using lower body positive pressure (LBPP). **Design and Methods.** Fourteen males (age: 20.6 ± 2.4 years) performed bouts of walking in both simulated Mars gravity (0.38 G) and Earth gravity (1 G) using an LBPP device. Dependent variables were the muscle activity evoked in the tibialis anterior, vastus lateralis, gluteus maximus and lateral portion of the gastrocnemius, measured using electromyography and expressed as percentages of maximum voluntary isometric contractions, and heart rate (HR). For statistical analysis, a paired t-test was performed. Statistical significance was defined as $P < 0.05$. **Results.** No significant differences in muscle activity were found across conditions for any of the investigated muscles. A significant mean difference in HR was identified between Earth (105.15 ± 8.1 bpm) and Mars (98.15 ± 10.44 bpm) conditions ($P = 0.027$), wherein HR was lower during the Mars trial. **Conclusions.** The Mars environment may not result in any deteriorative implications for the musculoskeletal system. However, if future research should report that stride frequency and thus activation frequency is decreased, in simulated Mars gravity, negative implications may be posed for muscle retention and reconditioning efforts on the Red Planet.

Keywords: Mars, walking, hypogravity, lower body positive pressure, electromyography.

Introduction

The next big milestone in human space exploration will be stepping foot on Mars. Current projections suggest a round trip to the Red Planet will take close to thee years, with approximately 12 months being spent in microgravity travelling to and from Mars, and 26 months on the Martian surface. At present, no human being has spent longer than 437.7 days (14.4 months) continuously in microgravity. When muscle and bone are mechanically unloaded, degeneration of these tissues ensue[1]. For a Mars expedition crew, this could mean deconditioning to the point of debilitation. A meta-analysis by Narici and Boer (2011)[2] highlighted the anti-gravity and locomotor muscles as most susceptible to mass losses induced by spaceflight. Moreover, marked degrees of atrophy (≤ 15%) in these muscles have been documented after as little as nine days in microgravity[3, 4]. With regards to the skeletal system, maladaptation brings about appreciable losses in bone mineral density[5]. A decrease in intestinal calcium absorption and a rise in bone resorption has led to virtually every astronaut exposed to microgravity for longer than 30 days suffering bone loss[6]. Moreover, post-flight monitoring from Skylab revealed the crew continued to experience bone demineralisation for a further 5 years following their return to Earth[7]. Considering that musculoskeletal degeneration and the accompanying increase in astronauts' susceptibility to accident induced injuries and osteoporosis is evident after relatively short duration missions, a return to Earth following a 3-year Mars mission may be disabling in the absence of efficacious countermeasures.

It is essential to understand the musculoskeletal loading deficits that inhabitants would experience on Mars. Earth-based simulations of partial gravity environments can provide a platform to be employed for determining the degree of muscle activity (electromyography – EMG) relative to maximum that can be achieved, in addition to relative muscle comparisons[8].

The latest and least functionally limited hypogravity analogue to emerge is the lower body positive pressure (LBPP) box treadmill[9]. Enclosing volunteers' lower limbs inside an air-tight inflatable chamber, attached at the waist, the LBPP box utilises pressure differentials to generate lift and alter the weight of the participant as desired. While stood on a treadmill, gait and other physiological measurements can then be analysed in simulated hypogravity.

Using this technology mostly to assess its efficacy as a gait rehabilitation tool, a handful of studies have employed EMG in lower body musculature [10, 11, 12, 13, 14, 15]. All of these studies reported reductions in levels of muscle activity with bodyweight support. However, only one of these studies, Klarner et al. (2010) [11], explored muscle activity during walking, the type of locomotion that Mars inhabitants will spend most of their time performing, whereas Gluteus Maximus (GM) activity was not analysed. While numerous kinetic analyses of locomotion on Earth have identified low activity in the GM during steady-speed, level walking, it is known that this muscle also contributes greatly to spinal stabilisation[16, 17]. As postural muscles, the spinal stabilisers would likely atrophy during a return mission to Mars, therefore, any deficits in GM motor recruitment while walking on Mars could have significant implications.

Evidence shows the cardiovascular system also experiences maladaptation with extended microgravity exposure, and several studies have also analyzed and compared heart rate (HR) at varying levels of simulated partial gravity. A convergent trend to this research is that HR decreases with increasing bodyweight support from an LBPP box. Jensen et al. (2009)[12] and Ruckstuhl et al. (2010)[18] both reported significant HR declines from 1G to running at 20% bodyweight and walking at 33% bodyweight, respectively. Cutuk et al. (2006)[19] also found HR to decrease during LBPP walking, while Nishiyasu et al. (2007)[20] observed HR to reduce with subjects simply standing upright with LBPP. More recently, Schlabs and colleagues (2013)[9] reported that HR was elevated to a significantly greater degree during walking in simulated

Mars gravity compared to simulated Moon gravity using an LBPP box. It thus may be expedient to include HR as a dependent variable in any prospective EMG studies related to Mars to gauge the degree of cardiovascular deconditioning that may occur in astronauts.

The main purpose of this study was to determine the influence of simulated Mars gravity, using an LBPP box treadmill, on the activity evoked in four locomotor muscles – Tibialis Anterior (TA), Vastus Lateralis (VL), Gastrocnemius Lateral (GL) and Gluteus Maximus (GM), compared to 1G while walking. A secondary aim was to analyze and compare HR between conditions.

Methods

Participants

Fourteen healthy males (age: 20.6 ± 2.4 years (mean ± standard deviation (SD)); height: 171.9 ± 4.4 cm; body mass: 65.2 ± 8.2 kg) volunteered to participate in this study. Contraindication to participation included any physical condition that could affect gait pattern or walking. The study was approved by the ethics committee of The Pontificia Universidade Catolica do Rio Grande do Sul (PUCRS) and conformed to the Declaration of Helsinki. Informed consent was obtained from all volunteers prior to testing.

Experiment Design

Participants attended one session at the John Ernsting Aerospace Physiology Laboratory at PUCRS, Porto Alegre, Brazil, where they performed bouts of walking in both ‘Mars’ (0.38 G) and Earth (1G) conditions inside the LBPP box treadmill. In this experiment, the level of muscle activity evoked in the TA, GL, VL and GM was measured using EMG, and HR (BPM), while walking on an LBPP box treadmill at a pre-determined pace that corresponded to a rate of perceived exertion (RPE) of 9 on the Borg scale (6 to 20 [AU]) [21] to ensure locomotive intensity was relatively equivalent. Testing was conducted in a randomised and counterbalanced repeated measures design to mitigate the influence of order effects[22]. Maximum voluntary isometric contractions (MVIC) of all investigated muscles were performed at the beginning of each session to provide normalisation values for the raw EMG data and allow comparisons between muscles regarding their relative degree of activity [23].

Electromyography Procedures

Disposable silver-silver chloride (Ag Ag – Cl), bipolar configured passive wet gel surface electrodes (Covidien llc, Mansfield, MA, USA) were affixed in pairs, spaced 20 mm apart, to the belly of each muscle of interest on the right leg, in accordance with SENIAM (Surface electromyography for the non-invasive assessment of muscles) guidelines and oriented parallel to the muscle fibres [24] (Figure 1). To minimise noise signals and artefacts, the skin was shaved and cleansed with an alcohol wipe before electrode attachment. With participants each attending only one session, the reliability limitation of re-attaching electrodes in the same positions for each muscle between trials was accounted for.

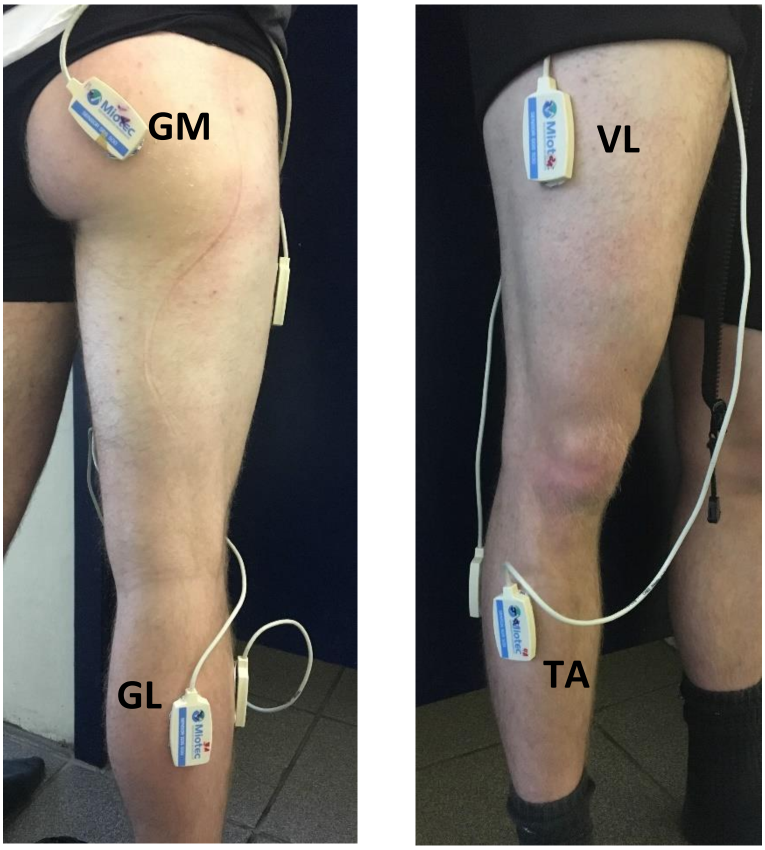

Figure 1. Displaying the electrode placement sites, based on SENIAM guidelines, for all four investigated locomotor muscles – Tibialis Anterior (TA), Vastus Lateralis (VL), Gastrocnemius Lateral (GL) and Gluteus Maximus (GM) (source – authors)

Attached to the back of the electrodes via snap fasteners, a wired telemetric module transmitted the myoelectric signals back to the 4-channel EMG system (MioGraph 4 canais, Miotec Equipamentos Biomedicos, Porto Alegre, RS, Brazil) where they were sampled at 2000 samples/second. A wired reference (ground) electrode was placed around the wrist (electrically neutral tissue) of participants using a bracelet to provide a common reference to the differential input of the preamplifier in the electrode. To optimize transmission, a low pass filter was applied to signals for smoothing and noise removal. All raw EMG data was saved using manufacturer's software (MioTool 2.0, Miotec Equipamentos Biomedicos, Porto Alegre, RS, Brazil) where subsequent analyses were performed. At this stage, the data was full-wave rectified.All MVICs were performed unilaterally to maximise output amplitudes, with the right leg being used to maintain consistency with experimental data[25]. When acquiring the MVIC for the GL, participants were asked to stand on their right leg, using a chair in front of them for support, and subsequently perform a maximum effort plantarflexion[26]. Due to equipment inaccessibility, the MVIC for the VL was performed with a modified version of a technique which has been previously substantiated greatly. This entailed the volunteer sitting on a sturdy chair, with their hip flexed to 90° and their knee flexed to approximately 60 degrees [27, 28]. With a rope tied around the volunteer's ankle and anchored behind them, a maximum effort knee extension was then instructed. For the TA, participants sat on a sturdy chair, their hip flexed to 90°, their knee at 140° and ankle at 90° at the moment of exertion. The right foot was strapped with rope that covered the metatarsals and phalanges. A researcher, stepping on the rope at both sides of the participant's foot to create tension, ensured that the foot was fixated securely and no movement occurred. Participants were then instructed to perform a maximum effort dorsiflexion [29]. When acquiring the MVIC for the GM, subjects assumed a prone quadruped position, maximally extending the right hip with the knee flexed to 90° [8, 27, 30].

LBPP Box Treadmill

The LBPP treadmill used in this study was devised and built by engineers at PUCRS. Akin to the Alter-G treadmill, it consists of a treadmill enclosed inside a plastic inflatable chamber (Figure 2). Transparent sides allowed for kinematics to be visually inspected, in addition to allowing researchers to ensure that electrodes and Velcro straps remained affixed. The dimensions of the LBPP chamber were: length: 2.3 m, height: 1.3 m and width: 0.97 m.

To achieve the desired unweighting of subjects for the Mars condition, the air pressure inside the chamber was elevated to a certain degree above ambient. Creating a pressure difference between the upper- and lower bodies in this way generates buoyancy, unloading the lower body of participants standing on the treadmill [10].

To mitigate escaping air from the chamber, participants wore skin tight neoprene shorts, fastened to the leg using Velcro straps for extra security. The top of the shorts was widened and fitted with one half of a zip fastener, extending out to interlock with the second half of the zip teeth on the neoprene ring at the top of the chamber, sealing the participant in at the waist.

The LBPP box had a motor (blower) with a nominal frequency of 60Hz (max: 70Hz, min: 5Hz). Spinning at 2850RPM, it provided 800 W of power. Earlier experiments were conducted to determine the characteristic curve of the blower (pressure x flow rate) [31]. To control the frequency converter, the microcontroller used an analogue output that generated signals ranging from 0V to 10V used by the frequency inverter (to adjust the frequency of the motor between 5Hz and 70Hz). To simulate the weight on Mars, a frequency of ~47.5Hz was used. However, the exact frequency depended on the mass of the participant. For instance, a frequency of approximately 47.5Hz was used for a subject with 60kg of mass. For the Earth trial, the equipment was set to 11Hz so as to just inflate the LBPP box and not suspend the participant.

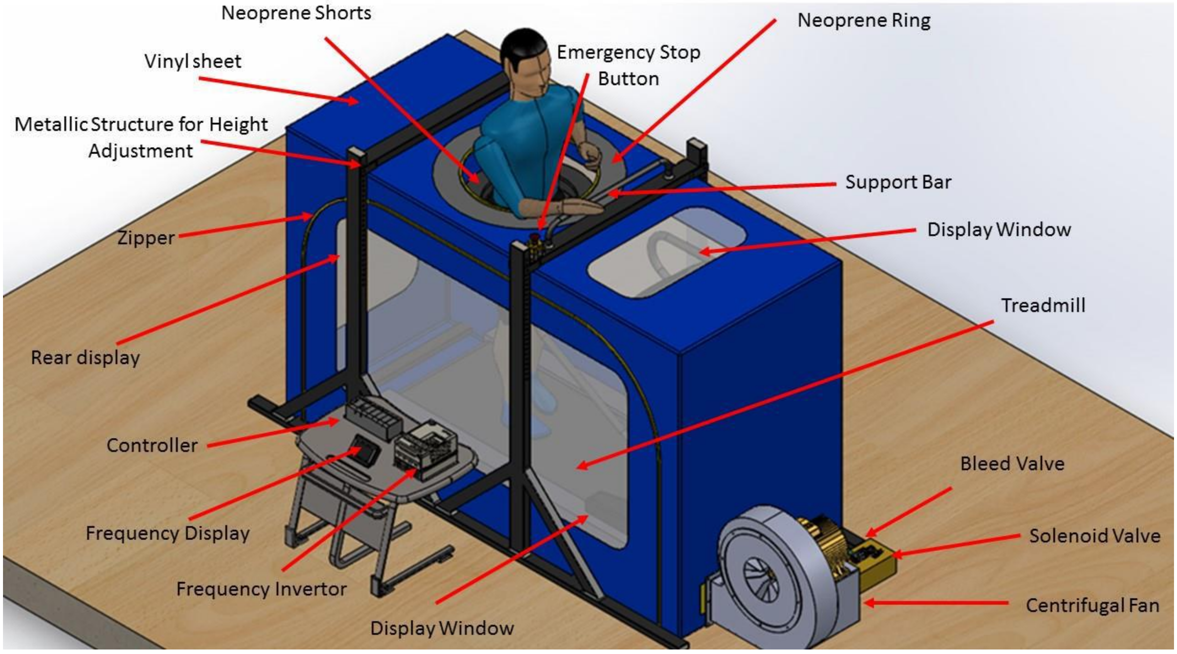

Figure 2. Annotated schematic of the LBPP device employed. (Source – Authors)

Experiment Procedures

After providing informed consent, all participants were weighed and heights measured without footwear. A research team member then prepared the skin surfaces at the sites of electrode placement and subsequently affixed the electrodes in place, according to SENIAM guidelines (Figure 1). After connecting the telemetric modules, participants donned the neoprene shorts and MVIC procedures began. After the ten-minute rest interval following the final MVIC, participants entered the LBPP device to stand on the treadmill and were then sealed inside the chamber with the zip fastener. The leads that transmitted the myoelectrical signals from the telemetric modules were guided underneath the neoprene shorts and out of the chamber to be connected to the EMG system. At this stage, the reference electrode was placed around the wrist of participants and connected to the EMG system. The chamber was then inflated according to the selected condition. Load cells underneath the treadmill translated the weight of participants to a digital display so that simulated Mars gravity at 0.38G could be verified against calculations from participants' pre-determined mass. Due to the LBPP system taking up to two minutes to stabilise for the Mars condition, with the subject standing in the chamber throughout, the same two-minute duration of standing was administered for the 1 G trial before experimental walking began to maintain consistency. When the target weight had been achieved following this two-minute period, basal HR was measured using a pulse oximeter. The treadmill was then started, increasing speed until communicated by the subject that an RPE of 9 had been reached. At this point, data collection began and EMG signals were collected for 3 min. HR values were taken again at 1.5 min and at 3 min, when the experiment was terminated.

Statistical Analyses

Data handling was performed using Microsoft Excel for Windows (2016), where mean (±SD) values were calculated for EMG data from all four muscles in both conditions for each subject, and then averaged across subjects. Following this, the data was imported into IBM SPSS 23.0 software for Windows (IBM, 2015) for inferential statistics to be performed on the data to determine whether any significant mean differences were present between conditions. Assessment of the normality assumption was first carried out using the Shapiro-Wilk test and a visual inspection of histograms before inferential statistics were performed. After verifying a normal distribution of data, a paired t-test was performed. In instances where data was assumed to be non-parametric, the Wilcoxon signed-rank test was employed. Statistical significance was defined as $p<0.05$.

Results

Muscle Activity

All reported muscle activity represents one minute of data taken between 1.5min and 2.5min of the protocol. Due to the presence of extreme outliers that skewed the distribution of data, when performing inferential statistics, values from four participants were omitted from the GM data, as well as one participant from the TA data and two participants from the VL data. Analyzing the results of the myogram for the GM, the paired t-test identified no significant mean difference between Earth (47.78 ± 3.24 mV) and Mars (47.78 ± 3.25 mV) conditions ($P = 0.877$). For the GL, TA and VL, the results of the Wilcoxon signed-rank test also failed to identify a significant mean difference between the myoelectrical signals elicited in Earth (47.32 ± 1.53 mV, 44.53 ± 7.06 mV and 50.66 ± 2.39 mV, respectively) and Mars (47.20 ± 1.54 mV, 45.50 ± 7.86 mV and 50.65 ± 3.03 mV, respectively) conditions ($P = 0.917$, $P = 0.182$ and $P = 0.722$, respectively) (Figure 3).

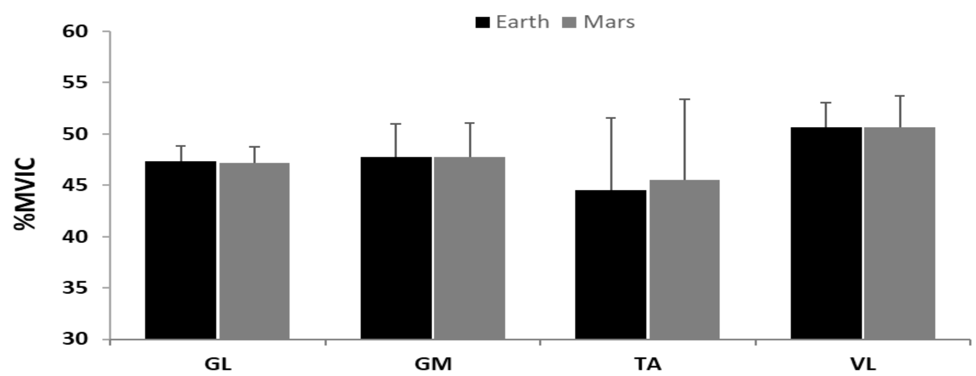

Figure 3. Muscle activity (mV) (mean and SD) expressed as a percentage of MVIC in the GL, GM, TA and VL during both conditions.

2.1. *Heart Rate*

Heart rate data represents the values recorded immediately preceding experiment termination at three minutes into each trial. The paired t-test revealed significant mean differences between Earth (105.15 ± 8.1 bpm) and Mars (98.15 ± 10.44 bpm) conditions (P = 0.027), wherein HR was higher during the Earth trial (Figure 4).

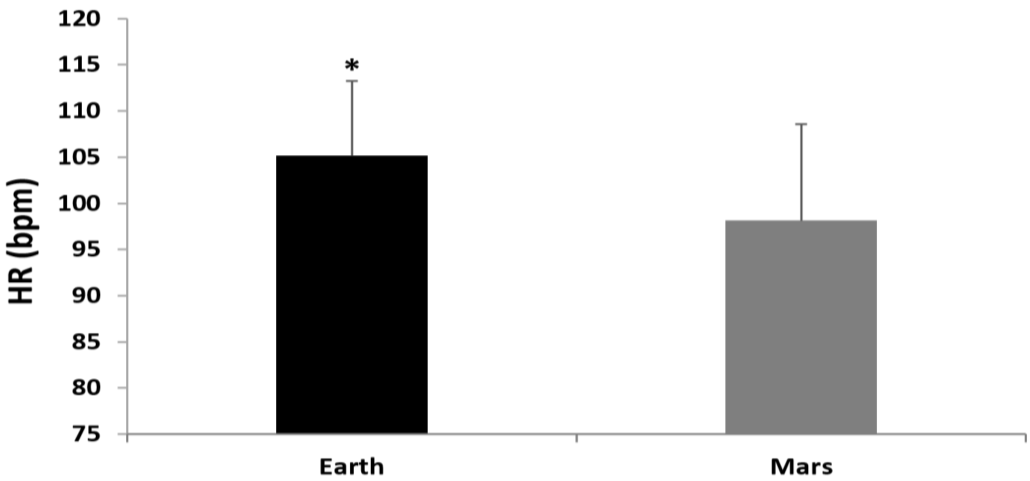

Figure 4. HR (bpm) (mean and SD) in both Earth and Mars conditions. *Significantly greater than Mars ($P < 0.05$).

Discussion

The main finding of this research was that no significant mean differences were identified between Earth and simulated Mars gravity conditions in any of the investigated muscles (Figure 3). This is in direct opposition to the only other existing piece of literature investigating locomotor muscle activity during walking in partial gravity similar to that on Mars[11]. However, the main focus of their research was to explore muscle coordination patterns with varying stride frequencies, and as a result, the exact degree to which muscle activity was attenuated with a 60% bodyweight reduction was not documented to enable complete comparisons to be drawn.

Our findings also contrast with the few running studies conducted in this area [10, 11, 12, 13, 14, 15]. A noteworthy finding of Hunter (2014) [10] was that hip adductor activity during the swing phase and medial and lateral hamstring activity during the first half of the stance phase were relatively unaltered with various levels of bodyweight support. This finding for the hamstrings has been substantiated elsewhere in the literature in the biceps femoris [12, 13]. While this may be perceived as somewhat supporting our findings, since these muscles are also instrumental in locomotion, Hunter (2014)[10] postulated that this was perhaps due to participants being unaccustomed to ambulation with LBPP and that with more familiarisation and training, a significant effect may have been observed. However, seemingly, bodyweight supporting muscles or 'anti-gravity' muscles respond differently than muscles that are associated with acceleration, deceleration and stability linearly and rotationally. In this regard, the biceps femoris is primarily responsible for leg swing, while the hip adductors likely function is to keep the swinging leg moving perfectly in the sagittal plane with little medio-lateral deviation, and less contribution to supporting bodyweight during gait[32]. Theoretically, with more bodyweight support, only changes in potential energy are influenced, as opposed to kinetic energy, which is body mass dependent. Accordingly, muscles supporting the bodyweight during locomotion are more likely to exhibit a reduction in activity with LBPP, whereas muscles such as the biceps femoris should remain unchanged, assuming the movement pattern remains unaltered[12]. Indeed, it has been documented that increasing the speed of running using a fixed level of LBPP seems to augment overall locomotor muscle activity[12]. These findings greatly highlight the need for further investigations using walking as the chosen mode of locomotion with LBPP, to contribute to this almost non-existent body of literature and allow true comparisons with the findings of the current study, since prospective Mars astronauts will spend most of their time walking, as opposed to running on the Red Planet. Moreover, it has been suggested that due to running being less energy efficient than walking, i.e., more metabolically demanding, muscle activity may be relatively more reduced during unloaded running than during unloading walking [33]. Therefore, most of the existing literature in this area may be overestimating the muscle activity deficit that may occur during everyday ambulation on Mars; our findings serve to substantiate this notion.

Sainton and colleagues (2015)[15] found an overall reduction in lower limb activity with bodyweight reduction but reported that pre-contact and braking phase extensor activity exhibited no change. While this was a running focused study and the lowest simulated partial gravity was 0.6G equivalent, as mentioned, running may be more likely to evoke significant reductions in muscle activity during unloading, thus offering the possibility that a walking trial at 0.38 G may have generated similar results. Should this be the case, it may offer support to our findings, since VL activity was indifferent between conditions (Figure 3). However, unfortunately, 3D motion capture for kinematic gait analysis was not used in the current study and so it was not possible to correspond muscle activity with the different phases of the gait cycle to support this. Adding credence to the notion that muscle activity may be reduced to a relatively greater extent during unloaded running than during unloaded walking due to energy efficiency differences, Ruckstuhl et al. (2010)[18] reported that greater treadmill speeds elicit relatively greater declines in HR and oxygen consumption with unloading.

Interestingly, we found that HR was significantly lower in the Mars condition than in the Earth condition (Figure 4), substantiating all previous work [9, 12, 18, 19, 20]. In a process that is present during microgravity exposure, it is likely that the LBPP translocates blood from the lower body to the upper body, promoting venous return, reducing the baroreflex-mediated enhancement in sympathetic activity and lowering HR [34, 35]. However, the state of the cardiorespiratory system on Mars is not well simulated using LBPP as it only affects the lower body; as such, it may have confounding influences on cardiorespiratory variables.

It is also conceivable that, at any given treadmill speed, an increased stride time or stride length as evidenced by several LBPP studies, would demand less from the locomotor muscles over the same period, even in the presence of unaltered levels of muscle activity, since fewer steps are taken [12, 36]. As such, the metabolic demand would ostensibly be lower, requiring less work from the heart.

The results of the current study suggest that walking on Mars may not elicit any deficit in muscle activity compared to walking on Earth. However, the fact that participants were unaccustomed to locomotion inside the LBPP device may have created an unnatural gait and negatively influenced results, and a familiarisation period might be required. A limitation of the current study is that kinematic analyses were not performed. In future, it would be expedient for studies to employ 3D motion capture to analyse kinematics and enable correspondence between muscle activity and different phases of the gait cycle after synchronisation, to more sensitively detect in which instants any potential deficits may be present[9, 12, 36, 37].

Some degree of muscle atrophy will occur on the outward journey to Mars while exposed to microgravity, specifically in locomotive and postural or anti-gravity muscles, such as those investigated in the current study[2]. Based on our findings alone, the partial gravity environment on the Red Planet itself may not carry any deteriorative implications for the musculoskeletal system. If this were to be true, the implementation of efficacious strategies that promote muscle hypertrophy and strength, such as a well periodized and comprehensive resistance training regime, with particular attention to anti-gravity muscles, while on Mars may be able to reverse the atrophy suffered on the outward journey, in preparation for further microgravity exposure during the return trip to Earth. If, however, it is determined by future research that a reduction in stride frequency is present in simulated 0.38 G, then it may prove difficult to support muscle retention and reverse the atrophic effect of the outward journey.

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Conflict of Interests:

Funding:

References

1. Lloyd SA, Lang CH, Zhang Y, Paul EM, Laufenberg LJ, Lewis GS et al. Interdependence of muscle atrophy and bone loss induced by mechanical unloading. J Bone Miner Res 2014;29:1118-1148.
2. Narici MV, de Boer MD. Disuse of the musculo-skeletal system in space and on earth. Eur J Appl Physiol 2011;111:403-420.
3. LeBlanc A, Rowe R, Schneider V, Evans H, Hedrick T. Regional muscle loss after short duration spaceflight. Aviat Spac Environ Med 1995;66:1151-1154.

4. Akima H, Kawakami Y, Kubo K, Sekiguchi C, Ohshima H, Miyamoto A et al. Effect of short-duration spaceflight on thigh and leg muscle volume. Med Sci Sports Exerc 2000;32:1743-1747.
5. Whedon DG, Rambaut PC. Effects of long-duration space flight on calcium metabolism: Review of human studies from skylab to the present. Acta Astronaut 2006;58:59-81.
6. Smith SM, Heer M. Calcium and bone metabolism during space flight. Nutrition 2002;18:849-901.
7. Tilton FE, Degioanni JJ, Schneider VS. Long-term follow-up of Skylab bone demineralization. Aviat Space Environ Med 1980;51:1209-1222.
8. Hammond B, Marques-Bruna P, Chauhan E. Electromyographic activity in superficial muscles of the thigh and hip during the back squat to three different depths with relative loading. Journal of Fitness Research 2016;5:57-67.
9. Schlabs T, Rosales-Velderrain A, Ruckstuhl H, Stahn AC, Hargens AR. Comparison of cardiovascular and biomechanical parameters of supine lower body negative pressure and upright lower body positive pressure to simulate activity in 1/6 G and 3/8 G. Journal of Applied Physiology 2013;115:275-284.
10. Hunter I, Seeley MK, Hopkins JT, Carr C, Franson JJ. EMG activity during positive-pressure treadmill running. Journal of Electromyography and Kinesiology 2014;24:348-352.
11. Klarner T, Chan HK, Wakeling JM, Lam T. Patterns of muscle coordination vary with stride frequency during weight assisted treadmill walking. Gait & Posture 2010;31:360-365.
12. Jensen BR, Hoygaard-Hansen L, Cappelen KL. The effect of lower body positive pressure on muscle activation during running, Proceedings of the XXII Congress of the International Society of Biomechanics, Cape Town, South Africa. InternationalSociety

of Biomechanics2009; 143. https://nexs.ku.dk/ansatte/?pure=da%2Fpublications%2Fthe-effect-of-lower-body-positive-pressure-on-muscle-activation-during-running(22abacc0-2528-11df-8ed1-000ea68e967b).html [Accessed 08 April 2019].

13. Jensen BR, Hoygaard-Hansen L, Cappelen KL. Muscle activation and estimated relative joint force during running with weight support on a lower-body positive pressure treadmill. Journal of Applied Biomechanics 2016;32:335-375.
14. Liebenberg J, Scharf J, Forrest D, Dufek JS, Masumoto K, Mercer JA. Determination of muscle activity during running at reduced body weight. Journal of Sports Science 2011;29:207-220.
15. Sainton P, Nicol C, Cabri J, Barthelemy-Montford J, Berton E, Chavet P. Influence of short-term unweighing and reloading on running kinetics and muscle activity. European Journal of Physiology 2015;115:1135-1179.
16. Bartlett JL, Sumner B, Ellis RG & Kram R. Activity and functions of the human gluteal muscles in walking, running, sprinting, and climbing. American Journal of Physical Anthropology 2014;153:124-154.
17. Lieberman DE, Raichlen DA, Pontzer H, Bramble DM, Cutright-Smith E. The human gluteus maximus and its role in running. The Journal of Experimental Biology 2006;209:2143-2155.
18. Ruckstuhl H, Schlabs T, Rosales-Velderrain A, Hargens AR. Oxygen consumption during walking and running under fractional weight bearing conditions. Aviation, Space, and Environmental Medicine 2010;81:550-554.
19. Cutuk A, Groppo ER, Quigley EJ, White KW, Pedowitz RA & Hargens AR. Ambulation in simulated fractional gravity using lower body positive pressure: cardiovascular safety and gait analyses. Journal of Applied Physiology 2006;101:771-777.

20. Nishiyasu T, Hayashida S, Kitano A, Nagashima K, Ichinose M. Effects of posture on peripheral vascular responses to lower body positive pressure. American Journal of Physiology 2007;293:670-676.
21. Borg G. Perceived exertion as an indicator of somatic stress. Scandinavian Journal of Rehabilitation Medicine 1970;2:92-98.
22. Soncin R, Pennone J, Guimaraes TM, Mezencio B, Amadio AC, Serrao JC. Influence of exercise order on electromyographic activity during upper body resistance training. Journal of Human Kinetics 2014;44:202-208.
23. Burden A. How should we normalize electromyograms obtained from healthy participants? What we have learned from over 25 years of research. Journal of Electromyography and Kinesiology 2010;20:1023-1057.
24. Gullett JC, Tillman MD, Gutierrez GM, Chow JW. A biomechanical comparison of back and front squats in healthy trained individuals. Journal of Strength and Conditioning Research 2009;23:284-292.
25. Bao S, Mathiassen SE, Winkel J. Normalizing upper trapezius EMG amplitude: comparison of different procedures. Journal of Electromyography and Kinesiology 1995;5:251-257.
26. Ahmad N, Taha Z, Eu PL. Energy requirement, muscle fatigue, and musculoskeletal risk of prolonged standing on female Malaysian operators in the electronic industries: influence of age. Malaysian Journal of Computer Science 2006;1:47-58.
27. Ekstrom RA, Donatelli RA, Carp KC. Electromyographic analysis of core, trunk, hip, and thigh muscles during 9 rehabilitation exercises. Journal of Orthopaedic and Sports Physical Therapy 2007;37:754-815.

28. Albertus-Kajee Y, Tucker R, Derman W, Lamberts RP, Lambert MI. Alternative methods of normalising EMG during running. Journal of Electromyography and Kinesiology 2011;21:579-586.

29. Siddiqi A, Arjunan SP, Kumar D. Improvement of isometric dorsiflexion protocol for assessment of tibialis anterior muscle strength. MethodsX 2015;2:107-111.

30. American Council on Exercise (ACE) (2006). Glutes to the max: exclusive ACE research gets to the bottom of the most effective glutes exercises [online]. Available at: http://www.acefitness.org/getfit/GlutesStudy2006.pdf [Accessed 08 April 2019].

31. Disuta L. Desenvolvimento de uma Câmara de pressão positive para estudos em fisiologia especial. Thesis Availabe at: http://tede2.pucrs.br/tede2/handle/tede/6499 [Accessed 14 April 2019].

32. Gazendam MG, Hof AL. Averaged EMG profiles in jogging and running at different speeds. Gait Posture 2007; 25(4):604-14.

33. Saibene F, Minetti AE. Biomechanical and physiological aspects of legged locomotion in humans. European Journal of Applied Physiology 2003;88:297-316.

34. Fu Q, Iwase S, Niimi Y, Kamiya A, Kawanokuchi J, Cui J, Mano T, Suzumura A. Effects of lower body positive pressure on muscle sympathetic nerve activity response to head-up tilt. Am J Physiol Regul Integr Comp Physiol 2001;281:778-862.

35. Hinghofer-Szalkay H, Kravik SE, Greenleaf JE. Effect of lower-body positive pressure on postural fluid shifts in men. European Journal of Applied Physiology 1988;57:49-54.

36. Leães R, Cambraia R, Bacim F,Dalmarco G, Calder A et al., *Development of walking pattern evaluation system for hypogravity simulation*. In Encyclopedia of Healthcare Information Systems. Information Science Reference 2006; Hershey, New York. Chapter 56, p. 440-445

37. Kondo T, Yamada S, Asai C, Matsuoka T, Tanimura D, Awaji Y et al . The influence of skeletal muscle pump function and strength on exercise capacity in patients with chronic heart failure. Journal of Cardiac Failure 2014; Vol.20 Issue10, Supplement p. S160